

THE RELATIVE VITAMIN A POTENCY OF THE CAROTENOID
PIGMENTS OF YELLOW CORN AND GRASS LEAVES
AS DETERMINED BY FEEDING CHICKS

by

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	3
Definitions of Vitamin A Used by Various Workers.	4
Vitamin A Requirement of Growing Chicks	6
Vitamin A Requirement of Hens	10
Vitamin A Deficiency of Chickens.	13
Growing Chicks	13
Adult Fowl	15
Sources of Vitamin A in Poultry Feeds	17
EXPERIMENTAL RESULTS	21
Experiment I.	21
Experiment II	27
DISCUSSION	35
CONCLUSIONS.	39
ACKNOWLEDGMENTS.	40
LITERATURE CITED	41

INTRODUCTION

The vitamin A potency of plant materials is due entirely to the carotenoid pigments they contain. In the case of leaves beta-carotene is responsible for practically all of the vitamin A potency. Since the amount of this pigment in leaves is easily determined it is possible to calculate the vitamin A potency of leaves on the basis that 0.6 microgram of beta-carotene is accepted as the International unit of vitamin A.

In the case of yellow corn two pigments contribute to its vitamin A potency, beta-carotene and cryptoxanthine. Theoretically cryptoxanthine has only one-half the vitamin A potency of beta-carotene. Since there is no practical method of making a quantitative separation of these two pigments in yellow corn it is not possible to calculate its vitamin A potency.

The object of this experiment was to determine the relative vitamin A potency of the carotene of grass leaves and the petroleum phasic fraction (cryptoxanthine and beta-carotene) of yellow corn.

Two previous investigations have been made on this problem. Kuhn and Grundmann (1934) made a chemical assay on four varieties of yellow corn. Below are the micrograms of

carotene and cryptoxanthine, respectively, found in 10 grams of fresh corn of various kinds:

Italian	0.007 and 0.046
Hungarian #1	0.005 " 0.046
Hungarian #2	0.005 " 0.070
Grits	0.005 " 0.025

These data would indicate that the carotenoid pigments of yellow corn supply approximately 55 percent as much pro-vitamin A as pure beta-carotene. Fraps, Treichler and Kemmerer (1937) determined the carotene content of several feeds spectrophotometrically and then conducted a biological assay, using rats, to compare the vitamin A potency of the feeds. They found that a gamma of the carotenoid pigments of yellow corn and alfalfa hay contained 1.4 and 1.6 International units of vitamin A per microgram of carotene. They made the following statement about their results: "The average number of Sherman-Munsell units per gamma of carotene was less for the two samples of yellow corn than it was on the alfalfa or peanut hay. This may be accounted for by the fact that the active pigments in yellow corn consist in a large part of cryptoxanthine (40 to 70 percent, according to the writer's results) which has a lower vitamin A potency than beta-carotene. However, there may be an appreciable error in the results because too few samples were used." If

a gamma of the carotenoid pigment of yellow corn has the potency of 1.4 International units of vitamin A potency compared to 1.6 International units of vitamin A for alfalfa hay then the carotenoid pigments of yellow corn are about 85 percent as efficient as the carotene of alfalfa which is known to be practically pure beta-carotene.

To determine the vitamin A potency of yellow corn and of oat grass leaves a biological technic using chicks as the experimental animals was decided upon. To make such an assay the minimum vitamin A requirements of chicks for maximum growth must be known. The work of Ringrose and Norris (1936) seemed to furnish this information most satisfactorily, therefore, it was considered desirable to base our procedure on their results and check their results under Kansas conditions.

REVIEW OF LITERATURE

A complete review of literature on vitamin A, related to poultry nutrition, was also undertaken in order to more accurately interpret experimental results and avoid any error in procedure.

Definitions of Vitamin A Units Used by Various Workers

In the literature at present, there is a distinct lack of uniformity in the meaning of a vitamin A unit. The most generally accepted unit at present is the 1934 International unit, which was adopted by the International Conference of the League of Nations health organization in 1934. It is defined as the vitamin A activity of 0.6 gamma (0.006 mg.) of the International Standard beta-carotene which is pure beta-carotene. The 1931 International unit is defined as the amount of vitamin A in one gamma (0.01 mg.) of a standard carotene solution which later was found to be only 0.6 as active as the pure beta-carotene. Therefore, the 1931 and 1934 International units are the same by definition. Other units used by the various workers are:

(a) The Sherman-Munsell unit is defined as the amount of vitamin A which, when fed daily, induces an average gain of 3 grams per week in young rats weighing 35 to 50 grams and whose growth has previously been arrested by a diet lacking in vitamin A. Sherwood and Fraps (1934) used a 6-day week to produce an average gain of 24 grams in 8 such weeks. This unit is converted into the International unit by multiplying by the factor 1.4. Sherwood and Fraps (1935) used the factor 1.2 to convert this unit into International

units.

(b) The U. S. Pharmacopoeia unit is the same as the International unit. It is used by the control division of the United States Department of Agriculture and is based on a sample of cod liver oil which contains 3000 units per gram. When the potency of an unknown substance is to be tested, one group of rats is fed this standard reference oil and another group is fed the unknown. The potency of the unknown is determined by the relative growth of the two groups of rats.

(c) The American Drug Manufacturers Association (A. D. M. A.) unit is, according to Jukes (1937), and Cruickshank (1935), converted into International units by multiplying by the factor 1.4. Record et al (1937) report that Frohring and Weno (1934) state that one microgram of carotene is equivalent to two A. D. M. A. units.

(d) The microgram of beta-carotene has also been used as a vitamin A unit; it is converted into International units by dividing by the factor 0.6.

(e) The blue unit of vitamin A used, Guilbert and Hart (1934), is defined as the amount of chromogen in 0.05 to 0.2 c. c. of chloroform solution which will give the faintest detectable blue color with 2 c. c. of antimony trichloride reagent. One blue unit in terms of vitamin A is equivalent

to 0.1 to 0.25 rat units; in testing carotene one blue unit is equal to 1.0 to 1.5 gamma of crystalline carotene. Therefore, one International unit equals approximately 10 to 25 blue units from vitamin A or 1.67 to 2.5 blue units from carotene.

In the literature here reviewed, all vitamin A units will be adjusted, in so far as is possible, to International units of vitamin A.

Vitamin A Requirement of Growing Chicks

Numerous research workers have made vitamin A studies on the chick, which have led to the now accepted fact that White Leghorn chicks require about 150 International units of vitamin A per 100 grams of feed for normal growth and prevention of all symptoms of vitamin A deficiencies, to 8 weeks of age. The chicks must have a vitamin A reserve when hatched to give these results, and this depends upon the hens having a normal diet containing ample vitamin A.

A number of early workers who established knowledge of the vitamin A requirement of the chick are: Emmett and Peacock (1922, 1923) fed chicks vitamin A deficient rations which caused them to develop ophthalmia in one experiment and terminated their lives in 14 to 21 days in another experiment. Hart, Halpin and Steenbock (1922) found that

chicks required a liberal supply of the fat soluble vitamins in cod liver oil. Mitchell, Kendall, and Card (1923) fed chicks on rations that were deficient in vitamin D and with varied amounts of vitamin A from yellow corn. They have confused vitamins A and D, but their data showed a significant difference between those pens which were receiving vitamin A and those which were not, indicating that vitamin A was essential. Hart et al (1924) found that either dried clover or yellow corn contained enough vitamin A for normal growth of chicks. Payne and Hughes (1933) found that chicks hatched from eggs laid by hens receiving various amounts of vitamin A in their ration lived on a vitamin-A-free ration a period of time proportional to the amount of vitamin A in the hen's ration.

The results of all experiments on the quantitative estimation of the amount of vitamin A required by the chick for normal growth and prevention of all signs of vitamin A deficiencies have been recorded in Table 1.

Other important facts established by the various workers are: Kline, Schultz and Hart (1932) proved that carotene served as a source of vitamin A for the chick and that xanthophyll did not. Sherwood and Fraps (1935) found that chicks hatched from eggs low in vitamin A require a much greater amount in their ration than those hatched from eggs

Table 1. Summary of previous studies on the vitamin A requirements of growing chicks.

Authority and date	Unit and source used by authority	International units per 100 grams of feed	Measurements of vitamin A	Remarks
Hauge Carrick Prange 1927	:25% yellow corn : :50% yellow corn :	:175 : :350* :	:Normal growth to :10-12 weeks :Normal growth to :maturity	: : : :
Kline Schultze Hart 1932	:0.03 mg. carotene: :per chick daily :0.05 mg. carotene: :per chick daily :	:113/100 gm. :feed* :8 plus units :per chick daily :	:Growth to 7-8 weeks : :Growth to maturity : :	:Insufficient amount : :Insufficient amount :for chicks depleted of :their storage
Smith 1933	:25% yellow corn :	:175* :	:Normal growth to :maturity	: :
Frohring Weno 1934	:65 A. D. M. A. :fish oil and :carotene	:200* : :	:Requirement of :chick at 8 weeks :	:Daily requirement of :chick at 8 weeks not :to 8 weeks
Guilbert Hinshaw 1934	:130 gamma of car- :otene per 100 gm. :mash :	:200 : : :	:No clinical symp- :toms of vitamin A :deficiency till :28th week	:Probably ample to 8 :weeks. Not minimum :amount to 8 weeks :
Sherwood Fraps 1935	:125 Sherman-Mun- :sell/100 gm. feed: : :150 Sherman-Mun- :sell/100 gm. feed: :Alfalfa meal and :yellow corn	:175 : : :200 : : :	:Prevent signs of :deficiency to 8 :weeks : : : :	:Absorbed yolks high :in vitamin A : :Absorbed yolks lower :in vitamin A. Basal :rations low in vit- :amin G
Ringrose Norris 1936	:150 Inter./100 :gm. feed : :100 Inter./100 :gm. feed :Carotene in oil	:150 : : :100 : :	:Maximum growth to :8 weeks : :Prevent all signs :of deficiency to 8 :weeks	:Carotene in oil mixed :with basal ration twice :weekly : :
Biely Chalmers 1936	:50 Inter. units :daily. Fish oil : : :	:190 : : : :	:Normal growth to :8 weeks : : :	:Vitamin A was fed as :cod liver oil directly :into the crop in a- :mounts sufficient to :supply 50 units daily
Wilson Schroeder Higgins 1936	:1200 Inter./pound :of feed. Fish :oil :	:264.3 : : :	:Required to 24 :weeks of age : : :	: : : :
Baird 1936	:125 Inter./100 :gm. feed. Fish :oil	:125 : :	:Requirements to 8 :weeks :	:N. H. Red chicks : :
Bearse Miller 1937	:87.5 to 175 Sher- :man-Munsell/100 :gm. ration :175 Sherman :Alfalfa	:122.4-244 : : :244 :	:Maximum growth to :8 weeks : :Normal growth to :maturity	: : : : :
Record Bethke Wilder 1937	:50 to 100 micro- :grams of carotene: : : :100 micrograms :carotene : : :	:80-160 : : : :120-200 : : :	:Prevent internal :and external symp- :toms of vitamin A :deficiency to 8 :weeks :Cure and prevent :symptoms in chicks :depleted of vitamin :A to 10-12 weeks of :age	:Prophylactic experiment : : : : :Curative experiment : : :

*It was assumed that a chick will eat 3.25 lbs. feed to 8 weeks and that yellow corn contained about 5 International units per gram.

high in vitamin A, and even then these chicks have a greater mortality. It is the opinion of the writer that both the chicks and the hens receiving low amounts of vitamin A also received low amounts of vitamin G in their rations, which probably had some effect on the results obtained. Holmes, Tripp, and Campbell (1936) assayed the livers of 8-week-old chicks receiving .25 percent and .5 percent sardine oil and found that the chicks receiving the higher amount stored four times as much vitamin A as the chicks receiving the lower amount.

Among the several workers reporting on the vitamin A requirements of chicks during their lives, there is a marked variation in results. This variation may be accounted for, in part at least, under one or more of the following statements:

1. Difference in standards used to measure the units required.

2. Variation in the vitamin A storage of the absorbed yolk. Sloan (1936) has suggested the operative removal of the yolk from day old chicks to be used in vitamin experiments and described the procedure.

3. Losses of vitamin A from feed during storage.

4. Variation in the degree of vitamin A deficiency of basal rations.

5. Variation in the basal diets as to the supply of all

the necessary nutrients. Randoin and Netter (1937) report that excess of vitamin B in a ration has a vitamin A sparing action.

6. Variation in the methods of feeding chicks the vitamin A potent material.

Vitamin A Requirement of Hens

The amount of vitamin A required by hens to produce eggs high in vitamin A is much greater than that required by chicks for growth. Vitamin A deficiency is shown in a number of ways. Holmes, Doolittle and Moore (1927) reported that the vitamin A content of the egg, egg production, weight of egg, fertility, hatchability, and viability of the chicks hatched were all improved by the addition of cod liver oil to rations deficient in vitamin A. Bethke, Kennard and Sassaman (1927) state that the eggs from hens fed cod liver oil or having access to blue grass pasture, contained approximately 5 times as much vitamin A as eggs from hens fed the basal diet, which contained 30 percent yellow corn, or the basal diet supplemented with alfalfa hay. DeVaney, Titus and Nestler (1935) found that the feeding supplements of cod liver oil increased the vitamin A content of pullets' eggs in proportion to the amounts fed.

Jukes (1937) reviewed the extensive work of Sherwood

and Fraps (1932, 1934, 1935) and summarized it as follows:
"The vitamin A requirements for egg production have been extensively studied by Sherwood and Fraps (1932, 1934, 1935). They express their results in Sherman-Munsell units, which have been converted to International units in this review. In their report, it was noted that a mash containing 20 percent ground yellow corn, supplemented with yellow corn as a scratch, failed to supply the requirements, since the vitamin A content of the yolk decreased from 28 units to from 7 to 11 units during the experiment. It was calculated that 6.3 units in the feed were required to produce 1 unit of A in the egg, and the maintenance requirement was estimated at 150 units per day. Dried alfalfa was stated to be insufficient to supply the requirements, and green feed was recommended in addition. In 1934, the same authors noted a decline in the vitamin A content of the egg yolk during the laying season, even when the hens received 630 units per day. The hens receiving 630 units of vitamin A supplied by dried alfalfa, laid about 15 percent more eggs than those receiving 320 to 480 units. However, it appears to the writer that the rations supplying the lower levels were probably deficient in lactoflavin. Four units of vitamin A in the feed were required to produce one unit in the egg. Green feed was again recommended as being necessary to sup-

plement the ration. However, it seems that the alfalfa meal used was of distinctly low potency (110 units per gram). Sherwood and Fraps (1935) continued the investigation and found that the vitamin A requirements for the formation of feathers seemed to be as high as the requirements for egg production, since molting hens did not store vitamin A even when fed liberal amounts. The author estimated that 340 units per day, or about 430 units per 100 grams of feed were required to keep the hens in good health and maintain a good production of eggs. Once again, it appears that the ration was somewhat low in lactoflavin."

Russell¹ found that a ration containing 485 International units per 100 grams of feed gave as satisfactory results as those containing higher amounts, with regard to egg production, hatchability and mortality. Parkhurst² states that the vitamin A requirement for laying hens is in the neighborhood of 750 International units per 100 grams of feed. Bearse and Miller (1937) found that 700 International units per 100 grams of feed in the hens' ration supplied sufficient vitamin A for maximum hatchability but possibly not sufficient for maximum production.

Holmes et al (1936) presented data showing that high pro-

1. Russell, W. C., Personal correspondence, May 26, 1936.

2. Parkhurst, R. T., Personal correspondence, May 26, 1936.

ducing flocks fed rations rich in vitamin A can maintain an adequate body reserve of vitamin A through the production period.

Vitamin A Deficiency of Chickens

Growing Chicks. Day-old chicks when placed on a vitamin A deficient ration grow fairly normally for the first two to four weeks depending on the amount of vitamin A stored in the absorbed yolk. Hinshaw and Lloyd (1934) report that chicks on a vitamin-A-free ration showed the first symptoms of deficiency on the twenty-seventh day and deaths occurred from the thirty-fourth to the fifty-sixth day. They, also, report that poults showed the first symptoms of deficiency on the twenty-fifth day and deaths occurred from the thirtieth to the forty-fourth day. Payne and Hughes (1933) fed hens on rations containing 0, 5, 10 and 15 percent alfalfa leaf meal and then fed the chicks hatched from eggs laid by these hens on vitamin-A-free rations and report that the chicks lived an average of 11.4, 17.5, 24.5 and 24.3 days, respectively. Other investigators have made similar reports indicating conclusively that the amount of vitamin A in the absorbed yolk of the chick prevents vitamin A deficiencies in the first few weeks of its life.

Elvehjem and Neu (1932) report that the first symptoms

of lack of vitamin A in the chick is the cessation of growth. This is followed in a few days by a staggering gait, and a general incoordination of movements, which Hughes et al (1929) state is due to the degeneration of the nerves, accompanied by emaciation, drowsiness, and a ruffled condition of the feathers. Hinshaw and Lloyd (1934) observed listlessness and an unsteady gait to be the first symptom to develop in poults followed shortly or accompanied by a tendency to sit with sagging wings, drooping head, and closed eyes. An increase of the lacrimal secretions, a swelling of the nictitating membranes and a slight nasal discharge were also early symptoms. The principal differences between poults and chicks under the influence of vitamin-A-free diets were that the condition of the chicks seemed more chronic and they appeared more nervous than the poults. Hinshaw and Lloyd (1934) made careful studies daily and observed ophthalmia to occur in 81.25 percent of the poults and 100 percent of the chicks studied. Other workers reported this symptom to be less dependable. There is often no appreciable decrease in appetite, due to vitamin A deficiency. After the development of definite vitamin A symptoms, death usually results in the next few days.

Post-mortem examinations of young chicks reveal pustule-like lesions in the mouth, pharynx and esophagus along

with the accumulation of urates in the kidneys and ureters and sometimes on the surface of the liver and spleen (Cruikshank, 1935). Hinshaw and Lloyd (1934), observing poults and chicks, reported pustules in the mouth, esophagus, and crop, excessive urates in the kidneys and ureters and a caseous exudate in the bursa of Fabricus were the most consistent post-mortem lesions. They observed the lesions in the bursa in 69 percent of the chicks and 88 percent of the poults examined. Other lesions of importance observed in chicks but not in the poults were an enlarged proventriculus, and urates in the abdominal and thoracic cavities.

According to Elvehjem and Neu (1932), the uric acid content of the blood of chicks affected by vitamin A deficiency may vary all the way from the normal, of about 5 mg., to about 44 mg. per 100 c. c. of whole blood. Sankaran (1936) fed a fowl twelve weeks on a vitamin A deficient diet and it had 375 mg. of uric acid per 100 ml. of plasma while a control fowl had 8 to 34 mg. Ackert, McIlvaine and Crawford (1931) obtained results which led them to conclude that there was a lowered resistance to the large roundworm, Ascaridia lineata, in chickens four to seven weeks of age, when kept for five weeks on a diet deficient in vitamin A.

Adult Fowl. A number of workers have studied the effect of vitamin A deficiencies on adult fowls. Beach (1924)

gave the first adequate description of the disease. He described external and post-mortem lesions very similar to those described above for chicks (with the exception of the bursa of Fabricus), but they are usually developed into more conspicuous lesions, due to greater resistance of the adult fowl to adverse conditions and therefore a longer period of life after bacterial infection takes place.

Sherwood and Fraps (1932) observed that it usually requires 135 days for adult birds to develop typical cases of A-type avitaminosis when fed deficient rations. Bushnell and Brandly (1929) observed that it required 185 days. They also stated that birds lacking in vitamin A lose weight, the comb becomes pale, the feathers ruffled, and progressive signs of muscular weakness follow. These noticeable effects are undoubtedly preceded by lowered hatchability, reduced egg production, and finally a cessation of production.

It has been shown by several workers that A-type avitaminosis in adult birds is definitely related to the improper nourishment of the epithelial linings of the body, the normal epithelium being replaced by a squamous, stratified, keratinizing epithelium which is very susceptible to bacterial infestation. Emerique (1936) studied the relationship between avitaminosis A and nitrogen metabolism and found that in A-type avitaminosis there seems to be a cessation of

the synthesis of specific proteins. These specific proteins are probably those essential for the building of normal epithelial tissue which will resist bacterial infestation.

Sources of Vitamin A in Poultry Feeds

The fowl's requirement for vitamin A may be supplied either by vitamin A from fish liver oil or by the carotenoid pigments containing the beta-carotene group, which Capper, McKibbin and Prentice (1931) proved was converted into vitamin A in the chick's body. There are four carotenoid pigments in plants that contain the beta-carotene group. They are beta-carotene, which contains two beta-carotene groups, alpha-carotene and gamma-carotene, and cryptoxanthine which contains only one beta-carotene group.

The liver oils of the salmon, turbot, sturgeon, halibut and cod fish (Lovern, Edisburg, and Morton, 1933) are excellent sources of vitamin A; however, there is a great variation in the number of vitamin A units in a gram of oil. Its potency depends on the original amount in the oil, the concentration of the oil, and the destruction of the vitamin A in the oil by oxidation. The first two difficulties are being rapidly overcome as the manufacturers are standardizing their oils and selling them on a guaranteed potency basis; but very little progress has been made toward pre-

venting destruction of the vitamin A content by oxidation when the oil is mixed in the feed. Marcus (1931) found that 85 percent of the vitamin A was destroyed in ten days after having been mixed in the feed. Fraps and Treichler (1933) found that 41 to 100 percent of the vitamin A of cod liver oil was destroyed in 2 weeks while the addition of 1 percent hydroquinine to the amount of 1 percent of the oil reduced the destruction of the vitamin A to 71 to 80 percent in 3 weeks. Hughes (1936) states that when cod liver oil is used as a source of vitamin A, the feed should be mixed fresh at the time of feeding.

All green grasses and legumes commonly used for poultry forage are excellent sources of provitamin A, carotene. Davis and Beach (1926) also report that yellow carrots are equal in value to the commonly used varieties of green feed as sources of provitamin A for poultry. Wood, Atkeson, Wellhouse, and Johnson (1935) analyzed fresh timothy, red top, white clover, and blue grass and found them to contain 308, 431, 339, and 245 International units per gram of dry material, respectively. The plant materials commonly used in mashes as sources of vitamin A are alfalfa leaf meal, dried grass meal and yellow corn. Considerable work has been done on the vitamin A potency of alfalfa leaf meal and yellow corn. Various investigators have reported the vita-

min A potency of alfalfa leaf meal to vary from 2 International units to 583 International units per gram of feed. Payne (1937) reports that dehydrated young grasses may contain from 583 to 1,083 International units of vitamin A potency per gram of material. He also states that carotene in young grasses may be preserved by the A. I. V. or molasses silage method and by mixing the dehydrated meal with condensed buttermilk. Smith and Milner (1934) examined the carotene of alfalfa and secured data indicating that it is all beta carotene. Karrer and Schlientz (1934) found the same to be true of the grasses. Hauge (1935) studied the destruction of carotene during the curing process of alfalfa hay and showed that enzymes are directly responsible for the destruction and that their action is accelerated by the sun's rays and high temperatures. Smith (1937) found that baled alfalfa hay lost 50 percent of its vitamin A potency from August to November, none during the next three (cold) months, and after twelve months contained only 25 percent of its original potency. Miller and Bearse (1934) found dehydrated alfalfa to have twice as much vitamin A potency as sun-cured alfalfa and 30 times as much as yellow corn.

Yellow corn is considered the second most practical plant source of vitamin A for poultry feeding, the green plant materials being first. Russell (1930) observed that

the more highly pigmented the yellow corn the greater its vitamin A potency; white-capped-yellow corn was only 50 percent as potent as yellow corn. Steenbock and Hart (1922), Hauge (1930), and Takahaski and Masuda (1937) report that the vitamin A potency of yellow corn is concentrated in the yellow endosperm and that the color of the pericarp has no effect on the vitamin A content. Millhouse, Koser, Rocke and Hetter (1928) studied the vitamin A content of the various structural parts and milling products of yellow corn and found that the greater part of the vitamin A potency is concentrated in the endosperm and hence is found in the gluten. Fraps (1931) studied the vitamin A potency of yellow corn and various yellow and white corn crosses and determined that the vitamin A potency was associated with the genetic factor for yellow. He found samples of corn involving 1, 2, and 3 genetic factors for yellow contained an average of 2.25, 5, and 7.5 Sherman-Munsell units of vitamin A per gram. He also stated that season and locality have some effect on the vitamin A potency content. An experiment conducted at Purdue University (1931) showed that there was no appreciable loss in the vitamin A potency of yellow corn when exposed to the air for 6 months. Sherwood and Fraps (1932) found that yellow corn lost approximately 33 percent of its vitamin A potency in $8\frac{1}{2}$ months. Holmes and Tripp (1933) also observed

a wide variation in the vitamin A potency of yellow corn.

EXPERIMENTAL RESULTS

Experiment I

The chicks for this experiment were hatched from eggs laid by the college flock of experimental Single Comb White Leghorn pullets that were receiving a ration containing 5,300 International units of vitamin A per 100 grams of feed from 10 percent dehydrated alfalfa meal. The chicks were wing banded and divided at random on the basis of vigor and lot weights into four lots of 24 chicks each. They were brooded for the first four weeks in a battery brooder of the conventional type with electric heating units and thermostatic control. From the fourth to the eighth week they were brooded on the floor in pens $6\frac{1}{2}$ by 8 feet; heat was supplied by electric hovers and sand was used as litter. Fresh water and the mash feeds were kept before the birds at all times.

Weekly individual weights, feed consumption records, and observations for symptoms of vitamin A deficiency were made for the experimental period of eight weeks (January 7 to March 4). The chicks were weighed at approximately the same hour each week and observations for signs of vitamin A deficiency were made at that time (two additional observations were made during the eighth week). An autopsy was

made on all birds that died during the experimental period by the poultry pathologist of the Department of Bacteriology of the college.

The vitamin A deficient basal ration used was essentially the one formulated by Ringrose and Norris (1936). It consisted of:

	Percent
White corn meal	55.5
Wheat shorts	25
Commercial casein	10
Dried yeast	5
Pulverized limestone	2.5
Steamed bone meal	1.0
Vitamin D concentrate	0.5
Salt	0.5

Vitamin D was supplied by a vitamin D concentrate³ in sesame oil, and used in such a quantity as to supply 100 U. S. P. units of vitamin D per 100 grams of feed. From the fourth to the eighth week the chicks also received the sunshine through vita-glass.

Yellow corn gluten meal and dehydrated oat leaves were

3. The vitamin D concentrate was supplied through the courtesy of Lancaster Co., Inc., Los Angeles, California.

used as the source of the test materials. The former is known to contain practically all of the carotenoid pigments of yellow corn and the dehydrated oat leaves were known to be a source of practically pure beta-carotene.

The two test materials were analyzed for their carotenoid pigment content by the method described by Peterson, et al (1937). It consists essentially of digesting the sample for 30 minutes with a saturated solution of potassium hydroxide in ethyl alcohol, adding petroleum ether, and washing the mixture with water to separate out the chlorophyllins, the flavones, and xanthophyll. The carotenoid pigment content of the petroleum ether phase is then determined by making optical density measurements at wave lengths of 4550, 4700, and 4800 Å on the spectrophotometer and applying the formula $c = D/kb$, where b is the thickness in centimeters of the layer of the solution, c the concentration of carotene in grams per liter, D the optical density and k the extinction coefficient. The yellow corn gluten meal contained 3.63 mg. of pigment per 100 grams of feed and the dehydrated oat leaves contained 39 mg. of pigment (beta-carotene) per 100 grams of feed. The dehydrated oat leaves were fed in such an amount to supply 100 and 150 International units (60 and 90 gamma of beta-carotene) of vitamin A per 100 grams of feed and each of the two yellow corn lots received

an equal quantity by weight of the carotenoid pigments of yellow corn. On the basis of these calculations it required 0.70 and 1.05 grams of dehydrated oat leaves and 7.5 and 11.3 grams of yellow corn gluten meal to supply the required 60 and 90 micrograms of the carotenoid pigments for each pound of feed.

The eight weeks' supply of feed was mixed at the beginning of the experiment and stored in iron barrels in the feed house. An analysis of the two test materials at the end of the experiment showed no destruction of the carotenoid pigments of yellow corn gluten meal and a loss of 25 percent of the carotene from the dehydrated oat grass leaves. No allowance was made in the data for this loss of carotene from the oat grass leaves.

During the fourth week of the experiment the experimental feed was sticking to the mouths of the chicks in an excessive amount, due probably to the yeast in the ration. To remedy this condition 3 percent wood pulp was added to the feed to increase its bulk and thereby decrease the sticky property.

The eight-week results of the first experiment are given in Table 2.

The symptoms used to diagnose the early stages of A-type avitaminosis were an enlargement of the eye ring, a

Table 2. Eight-week weights of chicks in experiment I fed various levels of carotenoid pigments of yellow corn and oat grass leaves.

Lot no.	Mgs. of pigment per 100 gms. of feed	Units of vit. A supplied	Number, average weight, and probable error of		Mor-tality	Chicks showing early symptoms of vit. A deficiency	
			Males	Females		Number	Percent
1	:Yel. corn 60	: ?	:15: 507.3+18.15	: 8: 568.1+14.40	: 1	: 14	: 69.6
2	:Yel. corn 90	: ?	:12: 542.5+20.23	:10: 542.5+12.71	: 2	: 3	: 13.6
3	:Oat grass 60	: 100	:16: 565.6+16.07	: 7: 557.9+12.94	: 1	: 3	: 13.0
4	:Oat grass 90	: 150	:14: 653.2+14.50	:10: 584.0+12.76	: 0	: 0	: 0.0

swelling of the nictitating membranes, an increased lachrimation and an unsteady gait. Post-mortem examination of ten chicks from each lot at the close of the experiment showed positive lesions of A-avitaminosis in only two chicks in lot three. These were small pustules in the esophagus.

The probable error of the mean was calculated by using the formula: $PE_m = \pm \frac{0.6745Q}{\sqrt{N}}$, where N is the number of variables and Q is the standard deviation. The standard deviation was calculated by use of the formula:

$Q = \sqrt{\frac{(v - M)^2}{N}}$, where (v - M) is the summation of the variables minus the mean, N the number of variables and Q is the standard deviation.

When analyzed statistically the data showed a significant difference between the weights of the males in lot 4 (90 gamma of pigment from oats) and the males of the other three lots, between the females of this lot and the females of lot 2 (90 gamma from corn), and between the males of lot 1 (60 gamma from corn) and the males of lot 3 (60 gamma from oats). There was no statistically significant difference between either males or females of lots 2 (90 gamma from corn) and 3 (60 gamma from oats), thus showing that 90 gamma of the carotenoid pigments of yellow corn have the same feeding value for vitamin A as 60 gamma of beta-carotene of oat grass leaves. Therefore, the yellow corn pigments are only 66.66 percent as potent in pro-vitamin A as

beta-carotene of oat grass leaves.

The percentage of chicks in each lot showing early symptoms of vitamin A deficiency lends support to the correctness of the above conclusion. The percentages were 13.6 for lot 2 and 13.0 for lot 3, while lot 1 showed 69.6 percent deficient chicks and lot 4 none.

There was an indication that the female chicks make better growth on vitamin A deficient rations than male chicks as shown by the data in Table 2.

Experiment II

The chicks for this experiment were hatched from hens and pullets of the same breeding as those used in experiment I. When eggs were saved for hatching this flock was receiving a ration containing 10 percent alfalfa leaf meal and 25 percent yellow corn in the mash, 50 percent yellow corn in the scratch grain and had access to young wheat pasture. It is estimated that their ration contained about 7,000 International units of vitamin A potency per 100 grams of feed.

This experiment covered the eight week period from April 5 to June 30.

The chicks were pedigreed, wing banded, and divided into eight lots of 25 chicks and one lot of 19 chicks; one chick from each hen was placed in each lot in so far as possible. They were brooded for the first four weeks in a

battery brooder kept in a room having a temperature of 90° the first week and 85° the remainder of the time. From the fourth to the eighth weeks they were kept in pens 4 by 9 feet. Sand was used for litter for the first three weeks and straw the last week. Water and the mash feeds were kept before the birds at all times.

The basal ration and test feeds used were the same as those used in experiment I. The carotenoid pigment content of the two test feeds was determined at the beginning of the experiment and again during the sixth week at which time the test feeds were mixed with the basal ration. The sample of dehydrated oat grass meal contained 19.36 mg. and 24.12 mg. of carotene and the sample of yellow corn gluten meal contained 3.61 mg. and 4.15 mg. of carotene and **cryptoxanthine** per 100 grams of feed at the beginning of the experiment and during the sixth week respectively. The higher carotenoid pigment content of the test feeds at the sixth week determination was due in part to the fresh sack of leaf meal opened and in part to an improvement in the technic of securing the pigment present. The mixed feed was stored in a basement where the temperature remained about 75° F. during the course of the experiment. Again no allowance was made in the data for loss of carotene from the experimental feed.

In this experiment lots 5, 6, 7, and 8 received 45, 60, 75, and 90 gamma of the carotenoid pigments of yellow corn. Lots 9, 10, 11, and 12 received 30, 45, 60, and 90 gamma of carotene from oat grass leaves; these amounts of carotene supplied 50, 75, 100, and 125 International units of vitamin A potency per 100 grams of feed. Lot 13 received the basal ration only. These amounts of pigment fed and the results of the second experiment are given in Table 3.

Eleven birds died during the first eight weeks of this experiment. The two birds which died in lot 9, died of A-type avitaminosis the day before the close of the experiment and one bird in lot 13 died 10 days prior to the close of the experiment from A-type avitaminosis. The other 8 birds died from causes other than A-type avitaminosis.

During the sixth week an outbreak of coccidiosis occurred in the chicks of lot 12, as was shown by their growth curve (Fig. I). A fecal examination showed the disease was also present in lot 8. Another fecal examination made three days later showed that the disease had spread to all lots. However, the disease apparently had no appreciable effect on the results of any of the lots except the two (8 and 12) mentioned above as shown by their growth curves (Fig. I). An autopsy of the chicks that died after the outbreak of coccidiosis showed that this disease was of major importance

Table 3. Eight-week weights of chicks in experiment II fed various levels of the carotenoid pigments of yellow corn and oat grass leaves.

Lot no.	Mgs. of pigment per 100 gms. of feed	Units of vit. A supplied	Number, average weight, and probable error of				Mor-tality	Chicks showing early symptoms of vit. A deficiency	
			Males	:	Females	:		Number	Percent
5	:Yel. corn 45	: ?	:10: 506.5 \pm 13.48	:	:14: 491.8 \pm 12.16	:	1	: 17	: 70.8
6	:Yel. corn 60	: ?	:10: 494.0 \pm 15.04	:	:13: 473.1 \pm 8.77	:	2	: 10	: 43.5
7	:Yel. corn 75	: ?	:13: 511.5 \pm 11.41	:	:12: 474.2 \pm 14.02	:	0	: 3	: 13.0
8	:Yel. corn 90	: ?	: 8: 496.3 \pm 14.64	:	:15: 475.3 \pm 9.94	:	2	: 15	: 60.0
9	:Oat grass 30	: 50	: 8: 420.6 \pm 23.03	:	:15: 458.0 \pm 11.07	:	2	: 18	: 78.3
10	:Oat grass 45	: 75	:15: 516.7 \pm 7.10	:	: 9: 544.4 \pm 17.13	:	1	: 4	: 16.7
11	:Oat grass 60	: 100	:12: 567.5 \pm 16.47	:	:13: 563.1 \pm 9.98	:	0	: 5	: 20.0
12	:Oat grass 75	: 125	:13: 498.5 \pm 12.22	:	:11: 481.8 \pm 14.30	:	1	: 7	: 29.2
13	:Basal 0	: 0	: 6: 410.8 \pm 17.22	:	:11: 398.2 \pm 10.65	:	2	: 16	:100.0

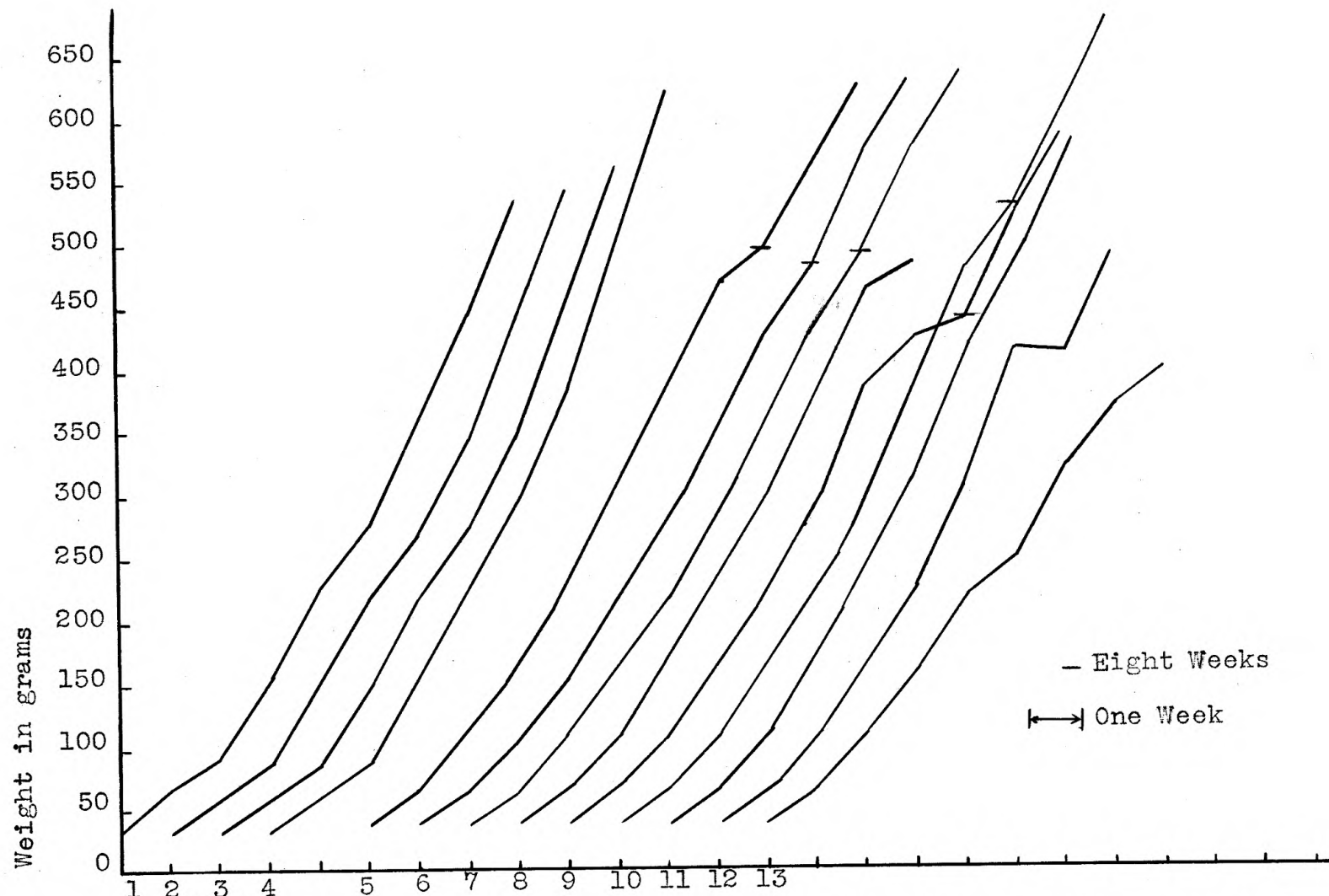


Figure I. Growth curves of lots 1 to 13 to eight weeks, and lots 5, 6, 7, 9, and 10 to ten weeks.

in only two of the chicks. The life cycle of the organism was apparently broken within a week by cleaning the pens daily and scalding the water utensils twice daily.

Lots 8 and 12 were not considered in drawing conclusions from experiment II as it was believed that coccidiosis significantly affected their growth.

The poor growth and the number of vitamin A deficient chicks in lot 13 showed that the basal ration was deficient in vitamin A. Their growth curve indicated that they had a large storage of vitamin A in their absorbed yolk, as they gained in weight as rapidly as the other lots until the fifth week.

Statistical analysis of the eight-weeks growth results in the other six lots of experiment II showed that there were no significant differences between any of the yellow corn lot, therefore, there was no basis for comparing any of the yellow corn lots with any of the oat lots. The results did show, however, that the lot receiving 100 units of vitamin A from oats was significantly larger than any of the yellow corn lots indicating that none of the yellow corn lots contain as much as 100 units of vitamin A. The eight-week results of the yellow corn lots were so irregular and showed no statistically significant difference, that lots 5, 6, 7, 9, and 10 were continued to 10 weeks of age.

The number of chicks showing vitamin A deficiency symptoms at 8 weeks appear to be a better guide to the value of the feed than their 8-week weights. These data indicate that the chicks which received 45 and 75 gamma of carotene from yellow corn gluten meal showed approximately the same number of vitamin A deficient chicks as those chicks receiving 30 and 45 gamma of carotene from oats. These results would indicate that the yellow corn pigments have between 60 and 66.66 percent as much vitamin A as the beta-carotene of oats.

Again the pullet chicks appeared to out-grow the cock-erel chicks on vitamin A deficient diets.

The results of the five lots of experiment II continued through the ten weeks are given in Table 4.

The weights of these five lots at ten weeks did not show a statistically significant difference between each oat lot and one or more corn lots, with the exception of the males in lot 10. They received 45 gamma of pigment from oats, and weighed (717.8 ± 23.09), their weight being significantly greater than any of the lots receiving the corn pigments. There was not a statistically significant difference between the weights of the males receiving 30 gamma of pigment from oats and those receiving 45 and 60 gamma of pigment from corn. Between the females receiving 30 gamma of pigment from oats and those receiving 45 gamma of pigment from corn,

Table 4. Ten-week weights of chicks in experiment II fed various levels of carotenoid pigment of yellow corn and oat grass leaves.

Lot no.	Mgs. of pigment per 100 gms. of feed	Units of vit. A supplied	Number, average weight, and probable error of				Mor-tality	Birds showing positive symptoms of vit. A deficiency	
			Males	:	Females	:		Number	Percent
5	:Yel. corn 45	: ?	: 9: 605.0+22.05	:	11: 635.9+13.87	:	5	: 12	: 60.0
6	:Yel. corn 60	: ?	: 9: 637.2+18.95	:	12: 610.8+11.06	:	4	: 14	: 66.6
7	:Yel. corn 75	: ?	: 12: 681.2+12.97	:	12: 595.8+23.12	:	0	: 6	: 24.0
9	:Oat grass 30	: 50	: 5: 550.0+27.76	:	14: 626.4+ 6.65	:	5	: 13	: 68.0
10	:Oat grass 45	: 75	: 14: 648.9+ 8.24	:	9: 717.8+23.09	:	2	: 9	: 39.0

and between the females receiving 45 gamma of pigment from oats and those receiving 60 and 75 gamma of pigment from corn. The insignificant difference between the various lots of males and females indicate that the pigments of yellow corn have $66\frac{2}{3}$, 50, $66\frac{2}{3}$, 60, and 75 percent of the vitamin A potency of the pigment, beta-carotene, of oat grass leaves.

The percentage of chicks in each lot showing positive symptoms of A-type avitaminosis agrees closely with the above results. The ten week weights showed that the females out-grew the males on vitamin A deficient diets.

DISCUSSION

The results of experiment I showed that 150 International units of vitamin A from beta-carotene in oat grass leaves were sufficient to give maximum growth and prevent all signs of deficiency to eight weeks of age. The chicks in lot 4 which received the 150 International units made slightly better gains than chicks from the same stock fed the regular college ration (containing ample vitamin A) and cared for in a similar manner until the sixth week after which time they were on range. The slightly larger size of the birds in lot 4 was probably due to their confinement.

This experiment confirms the results reported by

Ringrose and Norris (1936), and indicates that they may be satisfactorily used as a basis for further experimental studies of vitamin A under widely varied conditions.

The data did not show a statistically significant difference between the weights of either the males or females or both, of lots,

2 (90 γ of yellow corn pigment) and 3 (60 γ of oat pigment),
5 (45 γ of yellow corn pigment) and 9 (30 γ of oat pigment),
6 (60 γ of yellow corn pigment) and 9 (30 γ of oat pigment),
6 (60 γ of yellow corn pigment) and 10 (45 γ of oat pigment),
7 (75 γ of yellow corn pigment) and 10 (45 γ of oat pigment),

showing that the quantities of pigments fed these lots had the same vitamin A feeding value for chicks. When these quantities of pigments having the same vitamin A feeding value were converted into percentages and averaged they showed that the yellow corn pigments, cryptoxanthine, and beta-carotene, were 64.5 percent as potent in pro-vitamin A as the beta-carotene of oat grass leaves. This average percentage of 64.5 was the most accurate figure found to use in calculating the vitamin A potency of the carotenoid pigments of yellow corn when beta-carotene of dehydrated oat grass leaves was used as a standard. This figure was intermediate between the findings of Kuhn et al (1934) and Fraps et al (1937) being higher than the former and lower than

the latter. However, Fraps et al made only two biological tests with rats and one of their tests closely agrees with the results of this experiment.

During this experiment it was observed that the pullet chicks either weighed more or about the same as cockerel chicks on a vitamin A deficient diet. The chicks fed rations having just enough vitamin A to about prevent symptoms of A-type avitaminosis, the pullet chicks were equal in weight to the cockerel chicks, while in those lots receiving sufficient vitamin A the cockerel chicks consistently were larger than the pullet chicks, indicating sexual dimorphism in vitamin A requirement. This same relationship has been observed by other workers. Poulsson (1931) studied the investigations made by Birnbocker in connection with 330 cases of xerophthalmia of people in Vienna and it appears that only 38 or 11.5 percent of the cases referred to women. He also observed that this difference did not occur until sex differences occurred, which he placed at about five years of age in the human. He suggested that this may be due to the reserve of vitamin A in the female for use during pregnancy, and that the vitamin reservoir is probably the subcutaneous fat. He stated that the fat of bulls and cows varies in respect. Scheunert and Schieblich (1934) reported that the developed female herring contains more vitamin A than the

male. Murneck (1934) found that plants increased their carotene and xanthophyll content as they changed from a vegetative to a reproductive stage reaching their maximum content at the time of flowering. In dioecious plants, he indicated that there was predominantly more yellow pigments present in the female than in the male plant.

The results of this experiment have been influenced by three factors which should be carefully avoided by others using this method of assaying a feed for its vitamin A content. They are, 1. A variable and too great amount of vitamin A stored in the body of the day old chick; 2. Parasitism; 3. Too few chicks in each lot.

The first factor may be controlled by limiting the amount of vitamin A in the ration of the hen to just in excess of the minimum amount necessary for optimum egg production, hatchability and livability, and by hatching all of the chicks from these hens. The second factor may be controlled by keeping the chicks on wire floors. The last factor may be avoided by using a larger number of chicks in each lot. Variability among the experimental chicks could be reduced by pedigreeing and distributing each hen's chicks equally among the groups tested.

CONCLUSIONS

1. One hundred and fifty International units of vitamin A per 100 grams of feed were sufficient to give maximum growth and prevent all symptoms of A-type avitaminosis to eight weeks of age under conditions of this experiment.

2. The petroleum ether phase of the carotenoid pigments, beta-carotene and cryptoxanthine, of yellow corn has about 64.5 percent of the vitamin A potency of the beta-carotene of dehydrated oat leaves.

3. Pullet chicks excel or equal cockerel chicks in weight on vitamin A deficient rations and equal them on rations containing just enough vitamin A to prevent symptoms of A-type avitaminosis.

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